(19) European Patent Office

(11) Publication No.: **0 349 429**
A2

(12) **EUROPEAN PATENT APPLICATION**

(21) Filing number : 89401857.1

(51) Int. Cl.[5]: **A 61 K 9/127**

(22) Filing date : 6.28.89

(30) Priority : **6.30.88 FR 8808874**

(43) Publication date for application:
1.3.90 Bulletin 90/01

(84) Contractual countries specified:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: **CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS)**
15, Quai Anatole France
F-75007 Paris (FR)

(72) Inventors: **Serge Stainmesse**
8 rue Spinoza
F-94600 Choisy Le Roi (FR)

Hatem Fessi
9 rue Friant
F-75007 Paris (FR)

Jean-Philippe Devissaguet
14 Bd. d'Inkermann
F-92200 Neuilly sur Seine (FR)

Francis Puisieux
66 rue de Strasbourg
F-94700 Maisons-Alfort (FR)

(74) Represented by: **Peter Varady et al**
Cabinet Lavoix 2, Place d'Estienne d'Orves
F-75441 Paris Cedex 09 (FR)

(54) **A Process for Preparing Dispersible Colloidal Systems of Amphiphilic Lipids in the Form of Submicron Liposomes**

(57) A process for preparing dispersible colloidal systems of amphiphilic lipids, in the form of submicron oligolamellar liposomes whose wall is composed of lipids and potentially of a substance A and whose nucleus is composed of water or an aqueous solution, and may contain a substance B, characterized by the fact that: (1) a liquid phase is prepared that is composed basically of a solution of lipids and potentially of a substance A in a solvent or a mixture of solvents that can contain substance B in solution; (2) a second lipid phase is prepared basically composed of water or an aqueous solution of substance B: (3) the first phase is added to the second phase, with moderate agitation, to obtain a colloidal suspension of liposomes; (4) if desired, all or part of the solvent or mixture of solvents and water is eliminated, so as to obtain a colloidal suspension with the desired liposome concentration.
Applications: drugs, cosmetic products.

Description

A Process for Preparing Dispersible Colloidal Systems of Amphiphilic Lipids in the Form of Submicron Liposomes

The subject matter of this invention is a process for preparing dispersible colloidal systems of amphiphilic lipids in the form of submicron oligolamellar liposomes.

Many documents are known that describe the preparation and use of liposomes, particularly as vehicles of biologically active substances such as drugs, proteins, enzymes, diagnostic agents or cosmetic products. Thus, hydrosoluble substances can be encapsulated in the aqueous spaces of the liposome, or lipophilic substances can be incorporated in the wall of the lipid.

A process for preparing oligolamellar vesicular systems has already been described by Bangham and colleagues (J. Mol. Biol. 13, 238-252; 1965). According to this process, the lipids and lipophilic substances are dissolved in an organic solvent and treated with an aqueous phase with strong agitation. However, this process, like most of the known processes that are derived from it, does not make it possible to obtain directly liposomes with a particular size less than a micrometer, which would allow much greater stability of the particles and their dispersions.

The invention provides a simple process that can be used on a large scale to prepare submicroscopic-sized liposomes.

The invention therefore concerns a process of preparing dispersible colloidal systems of amphiphilic lipids in the form of submicron oligolamellar liposomes, whose wall is composed of said lipids and potentially of a substance A and whose nucleus is composed of water or an aqueous solution and may contain a substance B, characterized by the fact that:

(1) a liquid phase is prepared that is basically composed of a solution of amphiphilic lipids and potentially a substance A in a solvent or in a mixture of solvents, and may contain substance B in solution,

(2) a second liquid phase is prepared that is basically composed of water or an aqueous solution of substance B,

(3) the first phase is added to the second phase, with moderate agitation, so as to obtain, almost instantly, a colloidal suspension of liposomes.

(4) if desired, all or part of the solvent or the mixture of solvents and water can be eliminated, to obtain a colloidal suspension with the desired liposome concentration.

Substance A, which is lipophilic in nature, is designed to modify the physical or chemical characteristics (electric charge, rigidity) of the wall. It can be cholesterol, stearylamine, phosphatidic acid, alpha-tocopherol, a non-ionic surfactant, etc.

Substance B is the biologically active substance, particularly a pharmaceutical active ingredient or drug precursor, a biological reagent or a cosmetic product. Substance B is introduced into phase (1) if it is lipophilic and into phase (2) if it is hydrophilic.

The amphiphilic lipids can be glycolipids, phospho-aminolipids and particularly phospholipids, for example the lecithins (of egg, soy, etc.).

The solvent is preferable a water-miscible alcohol in all proportions, particularly ethanol.

The concentration of lipids in the solvent can be from 0.1% to 10% by weight, preferably 1% to 5% by weight.

It is an advantage if the volume of solvent used for phase (1) is composed of between 5% and 100%, for example around 50%, of the volume of water from phase (2), so as to obtain small-sized liposomes (particularly from 10 to 300 nm).

"Moderate agitation" is taken to mean agitation such as magnetic agitation, from 10 to 500 rpm, for example around 100 rpm.

Thus, the invention makes it possible to obtain drugs, particularly in injectable form, and cosmetic products that are very stable.

The following examples illustrate the invention.

Example 1: Preparation of Liposomes

Organic phase 1

Soy lecithin (Epikuron 170) 2.0 g

Absolute ethanol 50.0 g

Aqueous phase 2

Water 100.0 g

Phase 1 is added to phase 2 with magnetic agitation. The medium immediately turns opalescent with the formation of liposomes. The average size of the liposomes, measured immediately after preparation, in a laser beam diffractometer (Nanosizer^{R} from Coultronics) is 180 nm, with an average dispersion index of 0.5.

The alcohol is eliminated under reduced pressure, and the liposome suspension is filtered over sintered glass (pores 9-15 nm).

The size of the liposomes, measured again in the filtrate, remains unchanged.

A transmission microscope examination shows oligolamellar liposomes of a homogeneous size.

Example 2: Preparation of liposomes containing cholesterol (variation of Example 1).

Proceed as in Example 1, but add 0.30 g of cholesterol to the alcohol phase. The liposomes obtained have the same characteristics as in Example 1.

Example 3: Variation of Example 1.

Proceed as in Example 1, but replace the soy lecithin with egg lecithin. The liposomes obtained have the same characteristics as in Example 1.

Example 4: Variation of Example 2.

Proceed as in Example 2, but replace the soy lecithin with egg lecithin. The liposomes obtained have the same characteristics as in Example 1.

Example 5: Preparation of liposomes containing a hydrophilic active ingredient.

Proceed as in Example 2, but add 0.20 g of ampicillin (sodium salt) in the aqueous phase.

The level of incorporation of ampicillin in the liposomes, measured after separation of the liposomes from the aqueous phase by Sephadex gel chromatography, is 10%.

Example 6: Preparation of liposomes containing a lipophilic active ingredient.

Proceed as in Example 1, but add 66.7 mg of muramyl-tripeptide-cholesterol to the organic phase. The level of incorporation of the active ingredient is 100%.

Claims

1. A process for preparing dispersible colloidal systems of amphiphilic lipids, in the form of submicron oligolamellar liposomes, whose wall is composed of said lipids and potentially of a substance A and whose nucleus is composed of water and of an aqueous solution, potentially containing a substance B, characterized by the fact that:

(1) a liquid phase is prepared that is basically composed of a solution of said lipids and potentially a substance A in a solvent or in a mixture of solvents, and may contain substance B in solution;

(2) a second liquid phase is prepared that is basically composed of water or an aqueous solution of substance B;

(3) the first phase is added to the second phase, with moderate agitation, to obtain, practically instantly, a colloidal suspension of liposomes;

(4) if desired, all or part of the solvent or mixture of solvents and water can be eliminated to obtain a colloidal suspension with the desired liposome concentration.

2. The process in Claim 1, characterized by the fact that the amphiphilic lipids are phospholipids.

3. The process in Claim 1 or 2, characterized by the fact that said solvent is a water-miscible alcohol in all proportions.

4. The process in one of Claims 1 to 3, characterized by the fact that the alcohol is ethanol.

5. The process in any one of Claims 1 to 4, characterized by the fact that the concentration of lipids in the solvent is from 1% to 10% by weight.

6. The process in Claim 5, characterized by the fact that the concentration of lipids in the solvent is from 1% to 5% by weight.

7. The process in any one of Claims 1 to 6, characterized by the fact that the volume of solvent in phase (1) is composed of 5% and 100% of the aqueous volume of phase (2).

8. The process in any one of Claims 1 to 7, characterized by the fact that substance A is cholesterol.

9. The process in any one of Claims 1 to 7, characterized by the fact that substance B is a hydrosoluble drug.

10. The process in any one of Claims 1 to 9, characterized by the fact that the liposomes are approximately 100 to 300 nm in size.